EMBRYO SAC AND EMBRYO OF PENTSTEMON SECUNDIFLORUS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 248

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(WITH PLATE XII)

Two genera of the Scrophulariaceae were the first plants in which the development of the embryo sac and the embryo were correctly investigated. In 1851 HOFMEISTER (8), working on *Lathraea squamaria* and *Pedicularis sylvatica*, proved that the embryo was formed as a result of the fertilization of the egg, and not from the end of the pollen tube as was believed by SCHLEIDEN and his followers. DEECKE (5) in 1855 reinvestigated *Pedicularis sylvatica*. He insisted that HOFMEISTER was wrong and that the embryo really did develop from the end of the pollen tube. He was supported in his assertions by SCHACHT (11). Later, however, HOFMEISTER (9) proved that what DEECKE really saw was the proembryo. In his paper on *Lathraea* and *Pedicularis*, HOFMEISTER discusses the beginning of the endosperm and the haustoria. No further work of importance was done upon the Scrophulariaceae until 1874, when CHATIN (3) studied the development of the ovule and the seeds in a number of genera. Four years later VESQUE (14) worked on the embryo sac of a number of families, among which were included several of the Scrophulariaceae. Even as late as his publication of this paper VESQUE believed that SCHLEIDEN'S theory of the formation of the embryo was correct, and criticized HOFMEISTER'S interpretation as inaccurate.

One of the best contributions to our knowledge of the embryo sac situation in this family is by BALICKA-IWANOWSKA (2) in 1899. The account includes a study of a number of families of the Sympetalae, but deals especially with several genera of the Scrophulariaceae, particularly taking up the question of nutrition in the embryo sac. The haustoria are believed to have an absorptive power, and thus conduct nourishment into the embryo sac, the conclusions being based on the fact that the haustoria are always found in contact with parts which are well supplied with nourishment. Miss BALICKA-IWANOWSKA disagrees with HEGELMAIER (7) as to the function of the tapetum. HEGELMAIER believed it to act as a protective covering, while the former seemed to prove that it serves to pass nutritive substances on to the embryo sac, and that it possibly has a digestive function, since the cells of the integument adjoining it are found constantly breaking down. In 1906 SCHMID (12) investigated numerous species of the Scrophulariaceae. He discusses the formation of the embryo sac, fertilization, endosperm formation, and the development of the haustoria. He has done very little with the development of the embryo. In 1915 Miss MITCHELL (10) investigated the embryo sac and the embryo in *Striga lutea*, a semi-parasitic plant found in South Africa. In this form she has noted the lack of a tapetal layer.

The material for this study was collected near Boulder, Colorado, where the species is abundant. The indeterminate inflorescence affords flowers in all stages of development on the same plant. Such material was killed in chrom-acetic acid, cut in paraffin, and stained in various stains, safranin-gentian violet proving the best. Longitudinal sections 10 μ thick proved quite satisfactory for study.

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Ovary and embryo sac

The ovary of *Pentstemon secundiflorus* Benth. is of the ordinary bilocular scrophulariaceous type, with the partition somewhat swollen in the median line forming the placenta, which bears the numerous crowded anatropous ovules. Longitudinal sections of such an ovary at right angles to the partition afford a large number of ovules in each section for study. Sections of very young ovaries show the ovules beginning as slight swellings of the placenta. The megaspore mother cell is not distinguishable in such an early stage, becoming apparent only after the beginning of integument formation. It appears as a single enlarged and darker staining subepidermal cell, which functions directly. Growth is quite rapid and the cell soon becomes elongated. It is surrounded by a single layered nucellus. The single integument forms along the sides of the nucellus and soon surrounds it. A short time before the integument has completely surrounded the ovule the megaspore mother-cell has entered synapsis (fig. 1). Miss MITCHELL has estimated that about 10 per cent of the ovules of *Striga lutea* have reached synapsis at the same time. This percentage may safely be placed much higher for *P. secundiflorus*, probably more than 75 per cent of the ovules of a single ovary showing the same stage of development.

By the time the nucleus of the megaspore mother-cell has entered synapsis the young ovule is rapidly assuming its anatropous form, which is reached by the time the reduction divisions are completed. The first reduction division occurs about the time the integument has surrounded the ovule completely. This division is soon followed by the second, forming the row of 4 megaspores. Either the third or the fourth megaspore of the row may function in forming the embryo sac (figs. 2, 3). The other 3 disintegrate rapidly and become crushed by the growth of the one functioning.

The megaspore which functions increases rapidly in size, the micropylar end becoming bulbous while the chalazal end remains narrowed (fig. 4). The chalazal end, however, lengthens rapidly until it is 2–4 times as long as the bulbous portion. This growth carries it to a point in contact with the end of the vascular system. The nucellus early disappears, but by the time the embryo sac is formed another nutritive layer, the tapetum, has formed from the integument. During the growth of the embryo sac the single nucleus by 3 divisions has formed the 8-nucleate sac. The rapid growth of the sac causes the protoplasm to be much vacuolated.

In the earliest stage of the 8-nucleate sac 4 nuclei are found grouped at each end (fig. 4). Soon, however, a nucleus from each end migrates toward the opposite end. Eventually they meet and form the polar fusion nucleus (fig. 5). BALICKA-IWANOWSKA and SCHMID have commented upon the place of this fusion. The former says that it occurs near the middle of the sac, while the latter finds that it may occur anywhere in the sac of the Scrophulariaceae studied by him. In *P. secundiflorus* polar fusion was found to take place anywhere, seeming to be more a matter of chance than any regulated procedure. Regardless of where polar fusion takes place, the polar fusion nucleus is always found in the bulbous micropylar end of the sac at the time of fertilization (figs. 6, 7). It is here that the triple fusion is completed. By the time polar fusion is completed the egg apparatus is well formed and the antipodals have begun to disintegrate. In only one case were the antipodals observed to form anything resembling cell walls. The mature embryo sac (fig. 5) is one of the commonest stages of the sac to be found. This is probably due to failure to pollinate at once. A short period of inactivity always seems to occur.

The mature embryo sac is interesting in that it is always well filled with starch (figs. 6, 7). As soon as the megaspore begins its development into the embryo sac, traces of starch are to be found in it, although it is not until the embryo sac is well matured that large quantities of starch are present. Very often the adjacent tissues contain much starch also. After fertilization, when the endosperm and the embryo begin to develop, the starch in the sac disappears entirely. Many of the grains found in the sac are large, reaching 30 μ or more. Although starch is to be found in either end of the embryo sac it is always much more abundant in the micropylar end. SCHMID has found this to be true also.

False polyembryony

The fusion of 2 ovules appears to be a much more uncommon occurrence than the formation of 2 or more embryo sacs in a single ovule. Miss MITCHELL discusses a single case which she found in *Striga lutea*. The only other plants in which it has been reported are *Pyrus Malus*, *Loranthus europaeus*, and *Viscum album* (4). In the course of this study several cases in which 2 ovules had fused were noted. In some the fusion was quite complete, in others the ovule could be seen to be double. The presence of 2 micropyles as well as integumentary tissue between the 2 embryo sacs indicated that 2 ovules had fused. In one instance noted the egg apparatus had formed and polar fusion had occurred in both embryo sacs. False polyembryony seems to be quite common in this species.

Fertilization

In several cases the pollen tube with the tube nucleus and the 2 sperms were observed. While in the pollen tube the sperms are more or less capsule-shaped, but after reaching the embryo sac they become quite spherical. The pollen tube seems always to enter the embryo sac a little to one side, its entrance usually destroying one of the synergids, the other synergid disappearing soon afterward.

The sperms are readily distinguished from the egg nucleus and the polar fusion nucleus on account of their much smaller size. Fertilization of the egg and the triple fusion always occur in the micropylar end of the sac and in a normal manner. Both fusions occur at approximately the same time.

Several cases of double fertilization were observed (fig. 7). Previously double fertilization has been announced as occurring in *Digitalis purpurea*, *Linaria vulgaris*, *Melampyrum sylvaticum*, *Lathraea squamaria*, *Pedicularis foliosa*, and *Striga lutea* of the Scrophulariaceae. This adds *Pentstemon secundiflorus* to the list.

Formation of endosperm

Without resting after the fusion with the sperm, the endosperm nucleus by a series of divisions forms a large number of nuclei, which migrate to the chalazal end of the sac and there become peripherally placed. Simultaneous with the formation of the free endosperm nuclei the narrowed end of the sac begins to increase in size very rapidly, so that it soon surpasses the micropylar end in diameter (fig. 8). By the time the first endosperm walls have formed this end of the sac is much the larger. During all this increase in size a certain restricted area between the 2 ends remains very narrow, so that the embryo sac comes to be dumb-bell-shaped, with the chalazal end the larger. Endosperm walls continue to form in this end until the whole is completely filled (figs. 8, 10). Although endosperm nuclei are occasionally found in the micropylar end of the sac, no cell walls were observed to form. During endosperm formation the tapetum appears to be very active. Integumentary cells in contact with it are broken down and the tapetal cells are always filled with a dense protoplasm.

Haustoria

With the formation of the endosperm 2 large haustoria are formed: one in the neck which connects the micropylar and chalazal ends of the sac (fig. 8), the other as an outgrowth from the chalazal end of the sac (fig. 9). The former is formed by the growth of 2 endosperm cells forward through the narrowed neck and just into the micropylar end of the sac where growth stops. In the case of the chalazal haustorium there is an outgrowth of the sac in the region not covered by the tapetum. Into this bulbous pocket 4 endosperm cells grow. This brings the endosperm cells well into connection with the vascular tissue, the cells of which are gorged with nutritive material. The protoplasm of each haustorium is very dense. The cells of the chalazal haustorium are binucleate.

The active tapetal layer covers only the chalazal end of the sac (fig. 8), ending abruptly at its junction with the micropylar end. Schmid found that the tapetum might cover all of the embryo sac or only part of it as in *P. secundiflorus*. The latter condition seems the more common occurrence. Miss Mitchell found that no tapetum is formed in *Striga lutea*. She believes that this may be accounted for by the semi-parasitic habit of the plant.

Development of embryo

After fertilization the egg rests for a time, often even until endosperm cell walls have begun to form. It then divides, the first division being at right angles to the axis of the embryo sac. The segment nearest to the micropyle forms the suspensor, the other forming the embryo. By a series of divisions, coupled with rapid growth, the suspensor is transformed into a bulbous basal portion, and a number of smaller narrowed cells which lengthen rapidly in such a manner as to push the 1-celled embryo through the micropylar end of the sac (fig. 8) and into the center of the endosperm beyond (fig. 10). It is usually pushed from one-third to one-half of the way through the endosperm, where further progress is probably stopped by the division and growth of the embryo. The first 2 divisions of the embryo are at right angles to each other and in the plane of the long axis of the sac. The next division is at right angles to the first two and forms the 8-celled stage of the embryo. The 16-celled stage is formed by the periclinal division of the cells of the octant. The further division of the embryo was not followed.

After the embryo becomes imbedded in the endosperm the micropylar end of the sac, together with the suspensor, collapse and disappear. Their disappearance is accounted for by the pressure within the ovule, due to the increase in amount of endosperm which eventually comes to occupy all the space inside the seed coat.

Discussion

In the formation of the embryo sac of *P. secundiflorus* there is nothing strikingly different from that of other species of this family which have been studied, but the shape of the mature embryo sac is peculiar. The very bulbous micropylar end, with the long, narrowed chalazal end, gives the whole embryo sac a club-shaped appearance. The chalazal part of the embryo sac is never more than half as wide as the micropylar end at the time of fertilization. The drawings of the embryo sac of other Scrophulariaceae by Balicka-Iwanowska, Schmid, and Mitchell show that there is a tendency toward this shape of embryo sac in the family, but none of those drawn are so striking in shape as that of the species under consideration. The distance between the end of the embryo sac and the end of the vascular system is at first marked. As the sac later derives a large part of its nourishment through the vascular system, this may account for the necessity of lengthening the sac until the end comes in contact with this source of food supply.

During the development of the embryo sac traces of starch can be seen within it, and in all cases, by the time fertilization occurs, large quantities of starch are present. Often it is so abundant that the nuclei within the sac are partially or entirely obscured. By the time the embryo has reached the endosperm the starch has all disappeared.

D'HUBERT (6) has made a study of fleshy plants with regard to the formation of starch in the embryo sac. He finds that starch is always present in the sacs of fleshy plants such as the Cactaceae, Mesembrianthaceae, Crassulaceae, Portulacaceae, etc. He has also found, however, that some non-fleshy plants show starch in the embryo sac. According to D'HUBERT this latter case seems to be the exception rather than the rule, and he believes that there is a relationship between the fleshiness of the plant and starch in the embryo sac due to the slowness of the phenomena before fertilization. This, however, receives very little attention from him; nevertheless it seems the more plausible theory. *P. secundiflorus* is not a fleshy plant, but, judging from the drawings which D'HUBERT has made of several fleshy plants, it has more starch in its embryo sac than any of those figured. It appears that while there is activity in the embryo sac very little if any starch is stored up. As soon as the embryo sac matures and becomes inactive just before fertilization, possibly due to delay in pollination, the stream of nourishment which has been coming in cannot be checked suddenly but keeps passing more and more nutrition into the inactive sac, where it is stored in the form of starch. Such a conclusion seems to be substantiated by the fact that activity in the sac brought about by fertilization soon reduces the amount of stored-up starch. BALICKA-IWANOWSKA (2) has also investigated the deposition of starch in the embryo sacs of several plants, and has concluded that starch is only found in the embryo sac when the tapetum is cutinized. This does not seem to be the case in *P. secundiflorus*, however, as the tapetum is undoubtedly not cutinized. Moreover, it covers only half of the embryo sac, as has been explained before. SCHMID (12) has found starch present in the integuments as well as the embryo sacs of a number of the Scrophulariaceae. He states that the starch is found throughout the embryo sac, but that sooner or later it is all translocated to the micropylar end, "wo die lebhaftesten Teilungen stattfinden."

The function of the tapetum seems to be one of nutrition, as has been suggested by BALICKA-IWANOWSKA (2). That it may have a protective function, as has been suggested by HEGELMAIER (7), seems rather doubtful. This seems all the more questionable when one considers that it covers only the chalazal end of the sac in a number of species. Surely the micropylar end would be as much in need of protection. In *P. secundiflorus* the integumentary cells border on the micropylar end of the sac.

The two haustoria function in passing nourishment to the endosperm cells which are farther from the supply of food. By the time the embryo has reached the endosperm the micropylar haustorium becomes inactive and is lost. The chalazal one, however, functions until the endosperm is formed. The nuclei in this haustorium are very pronounced. On account of the large size and seeming activity of haustorial nuclei some authors have attributed to them a considerable rôle in nutrition. BALICKA-IWANOWSKA (2) has always found them near the point where nutrition is most abundant. In this work a similar tendency was noted.

The growth of the suspensor in such a manner as to push the proembryo through the micropylar end of the embryo sac and to imbed it in the endosperm is rather unique. SHARP (13) in his study on *Physostegia* has recorded a similar situation, but the method of endosperm formation in *Physostegia* and *Pentstemon* is different entirely.

During the growth of the suspensor which imbeds the embryo in the endosperm, nutrition is derived from the starch stored up in the micropylar end of the sac.

Summary

1. The embryo sac is developed from a single megaspore. Its antipodals disorganize early. The micropylar end becomes bulbous, while the chalazal end becomes long and narrow and is covered by a distinct tapetum.

2. The mature embryo sac is found to be constantly gorged with starch, due to the non-utilization of the nutritive materials which pass into the sac at a time of inactivity just before fertilization.

3. The endosperm nucleus immediately divides and free nuclei migrate into the chalazal end of the sac, where wall formation begins. The proembryo is pushed into this endosperm by an

extreme growth of the suspensor. The micropylar end of the sac disintegrates.

4. Two haustoria are formed, the micropylar by the growth of endosperm cells from the chalazal end into the micropylar end, and the chalazal by a growth of endosperm cells from the chalazal end out into the vascular system. The cells of the latter haustorium are binucleate.

5. False polyembryony occurs rather commonly in this species.

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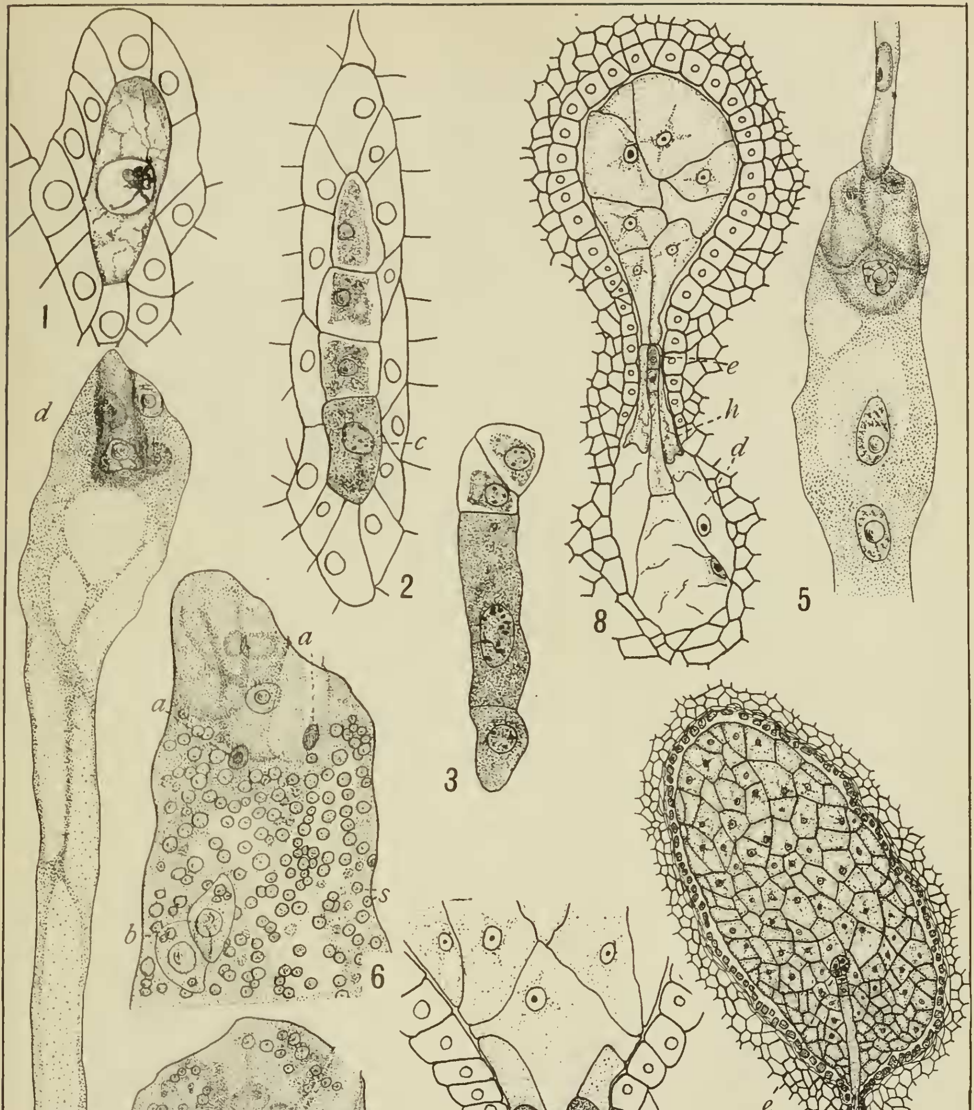

EVANS on PENTSTEMON